

Effect of Enteral Kanamycin on Experimentally Induced Ischemic Bowel Disease in Mice

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= Abstract = A mouse model using temporary occlusion of superior mesenteric vessels was tested for the effect of enteral antibiotics on the prevention and treatment of ischemic bowel disease. Ischemic bowel disease created by the temporary occlusion of mesenteric vessels was morphologically quite similar to the necrotizing enterocolitis (NEC) of the newborn. Non-absorbable Kanamycin administered enterally was shown to prevent the development of ischemic lesions, but its therapeutic effect was not established in this experiment.

Key Words: *Necrotizing enterocolitis, Enteral antibiotics, Ischemic bowel disease*

INTRODUCTION

On many pediatric intensive care units, necrotizing enterocolitis (NEC) became the most common newborn surgical emergency and has a mortality that far exceeds that of any other gastrointestinal condition requiring operation. Although its etiology is not known yet, its natural course has been well known to the clinicians. It is basically a disease of a very sick, small and vulnerable infants. Several risk factors (Kliegman *et al.* 1982) are known, i.e. hypoxia, low flow states, and bacterial invasion (Stoll and Kanto 1980). Initial mucosal injury by various risk factors can be followed by the entering of bacteria with its toxin into the bowel wall which will lead to the eventual necrosis of the intestinal wall. With earlier diagnosis and better selection of operative and nonoperative therapy, the mortality and morbidity of this NEC has been markedly reduced in recent years. Enteral antibiotics are being widely used as a way of treatment, though its effectiveness is still in question.

Krasna *et al.* (1986) developed a mouse model for the study of necrotizing enterocolitis using temporary occlusion of superior mesenteric vessels by "Bulldog" clamp for varying period of time. In order

to study the effect of bacterial flora on the etiology of NEC, non-absorbable Kanamycin was administered enterally to the experimental animal before and after the creation of ischemic bowel disease which resembled NEC of the human. The results of these experiment were presented.

MATERIALS AND METHODS

Studies were carried out in 275 five to six week old mice weighing about 18 to 20 gm. Animals were fed with routine animal food. Mice were anesthetized by the intraperitoneal injection of 1-2 cc of pentobarbital solution. With small midline incisions, the entire bowel was eviscerated from the abdominal cavity, and a microvascular bulldog clamp was applied to the superior mesenteric vessels for 10, 20 and 30 minutes respectively. Wound were closed as usual after the entire bowel was returned into the original position. Affected animals developed abdominal distension and tarry stool after 48 hours with subsequent death. Ischemic bowel lesions were noted to be either circumscribed area of gangrene or full thickness necrosis at the antemesenteric border similar to that of NEC. Most of the lesions were localized in the small bowel.

Enteral antibiotics: Non-absorbable Kanamycin was used for the test. Antibiotics were added into the drinking water of the animal at the concentration of 0.05 % (0.5 mg/ml). Animals were divided

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Table 1. Effect of enteral Kanamycin with 10 and 20 minute occlusion of mesenteric vessels on mice

group	n	Normal mice	Ischemic Bowel mice
10 minutes	50	47 [94 %]	3 [6 %]
20 minutes	50	21 [42 %]	29 [58 %]
enteral KM, pre-occlusion	50	42 [84 %]	8 [16 %]*
enteral KM, post-occlusion	50	28 [56 %]	22 [44 %]

KM: Kanamycin *: p < 0.01

into three groups: control group with varying occlusion time, group that received enteral antibiotics during the 48 hours prior to occlusion and group that received enteral antibiotics after the occlusion of superior mesenteric vessels. To encourage the drinking of Kanamycin-containing water, food was restricted for about 12 hours before and after the experiment.

RESULTS

Control Group

Control group was divided into two subgroups, according to the occlusion time. In the 10 minute occlusion group, only 3 of 50 mice showed ischemic bowel disease. In the 20 minute group, 29 of 50 mice ($p < 0.001$) developed bowel lesion (Table 1).

Enteral Antibiotic Treatment

For those animals who had been drinking Kanamycin containing water 48 hours prior to the occlusion of the superior mesenteric vessel, only 8 of 50 mice with 20 minute occlusion developed bowel lesions. Of 50 mice who had Kanamycin containing water after the occlusion, 22 developed bowel lesions. Effect of Kanamycin containing drinking water was statistically significant when it was given before the occlusion ($p < 0.01$). However, enteral Kanamycin given after the occlusion did not affect the progress or development of ischemic bowel disease in mice ($p > 0.10$).

Prolonged Occlusion Vs Enteral Antibiotics

A similar study was done to see the effect of antibiotics on prolonged occlusion (30 min.). The results are shown on Table 2.

DISCUSSION

Suppression of bacterial growth by the use of enteral nonabsorbable antibiotics has been used to

Table 2. Effect of enteral antibiotics with 30 min. occlusion of superior mesenteric vessels on mice

group	n	normal mice	ischemic bowel mice
30 min. occlusion	25	7 [28 %]	18 [72 %]
enteral KM pre-occlusion	25	14 [56 %]	11 [44 %]
enteral KM post-occlusion	25	9 [36 %]	16 [64 %]

KM: Kanamycin *: p < 0.01

prevent or treat NEC. There are contradicting reports (Boyle *et al.* 1978; Grylack and Scanlon 1978; Rowley and Dahlenburg 1978) for the value of enteral antibiotics in the prevention and treatment of NEC. The varying results appeared to be related to the fact that multietiological factors are responsible for the development of NEC. The available studies in human do not support the uniform effectiveness of the routine use of enteral antibiotics to the high risk premature infants.

In this study, ischemic bowel lesion can be created in mice with the occlusion of superior mesenteric vessels for 20 min. or more. The mouse model can be used for the evaluation of new therapy of NEC (Krasna *et al.* 1986). Enteral Kanamycin given prior to the occlusion of the superior mesenteric vessel prevented the development of ischemic bowel disease which was similar to the NEC of the newborn both microscopically or grossly ($p < 0.01$). But it failed to treat the developing lesion in both 20 minutes and extended occlusion groups ($p > 0.05$).

There is some gap for applying these results to the human NEC. The lesion created by occlusion of mesenteric vessels are similar to the NEC, but not identical. A six week old mouse is a grown animal compared to the human neonate. But the prevention of developing ischemic bowel lesion by enteral administration of Kanamycin in mice is quite striking. It also suggests that more than an ischemic factor is involved in necrotizing enterocolitis of the newborn. Enteral antibiotics for prevention of human necrotizing enterocolitis need further evaluation.

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= 국문초록 =

실험적 생쥐 허혈성장관질환에서 카나마이신 경구투여의 영향에 대한 연구

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김 우 기

치사율이 높고 합병증이 흔한 신생아 괴사성장염의 비수술적 치료의 한 방법으로 장관에서 흡수 안되는 경구용항생제 투여가 시행되고 있다. 이 경구항생제의 효과를 알아보기 위하여 18-20gm의 생쥐에서 실험적으로 소장간막혈관을 차단하여 허혈성장변화를 일으켰다. 이 허혈성장변화는 육안적으로나 현미경적으로 괴사성장염과 흡사하다. 이 생쥐를 실험모델로하여 경구 카나마이신의 예방효과와 치료효과를 비교관찰 하였다. 각각 20분, 30분의 혈류차단으로 10분 차단군에 비하여 유의있는 장병변이 발생하였으며, 혈류차단전 예방적으로 복용시킨 생쥐들은 통계적으로 유의있는 예방효과가 관찰되었으나, 치료효과는 없다는 것이 관찰되었다.

